

IN THE CLAIMS

1. (currently amended) A method for detecting a methylated cytosine in a template nucleic acid, the method comprising:

(a) providing a hairpin-template complex having a covalently attached 5' overhang, comprising:

(i) a hairpin nucleic acid, wherein the hairpin nucleic acid is self-complementary and has a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said recognition sequence is situated so that said cleavage site is before, at, or beyond the 3' end of the hairpin nucleic acid, and wherein said hairpin nucleic acid is a self-hybrid; and

(ii) a single-stranded template nucleic acid;

wherein the 5' end of the hairpin nucleic acid is covalently attached to the 3' end of the single-stranded template nucleic acid to produce a hairpin-template complex having a covalently attached 5' overhang;

(b) sequencing the single-stranded template nucleic acid of the hairpin-template complex having a covalently attached 5' overhang, thereby producing:

~~(i)~~ ~~(ii)~~ a first sequence; and

~~(ii)~~ ~~(i)~~ a hairpin-template-complement complex, comprising the hairpin-template complex having a covalently attached 5' overhang of (a), and further comprising a synthetic nucleic acid strand complementary to the template nucleic acid, wherein the synthetic nucleic acid strand complementary to the template nucleic acid is hybridized to the template nucleic acid, and wherein the synthetic complementary nucleic acid strand complementary to the template

nucleic acid is attached at its 5' end to the 3' end of the hairpin nucleic acid;

- (c) removing the synthetic complementary nucleic acid strand complementary to the template nucleic acid from the hairpin-template-complement complex, thereby recovering the hairpin-template complex having a covalently attached 5' overhang;
- (d) treating the hairpin-template complex having a covalently attached 5' overhang recovered in (c) with sodium bisulfite, thereby producing a sodium bisulfite-treated template nucleic acid;
- (e) sequencing the sodium bisulfite-treated template nucleic acid of (c), thereby producing a second sequence; and
- (f) comparing the first sequence and the second sequence, where the presence of a cytosine in the second sequence indicates that the cytosine at that position is methylated;

thereby detecting a methylated cytosine in the template nucleic acid.

2. (currently amended) The method of claim 1, wherein the hairpin nucleic acid is attached to a solid support substrate.

3-9. (canceled).

10. (currently amended) A method for detecting a methylated cytosine in a template nucleic acid, the method comprising:

(a) providing an anchor-template complex having a covalently attached 5' overhang, comprising:

- (i) a double-stranded nucleic acid anchor, wherein the double-stranded nucleic acid anchor comprises:
 - (A) a first end and a second end; and

- (B) a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said cleavage site is situated so that said cleavage site is before, at, or beyond the 3' end of the first end of the double-stranded nucleic acid anchor; and
 - (ii) a single-stranded template nucleic acid;wherein the 5' end of the first end of the double-stranded nucleic acid anchor is covalently attached to the 3' end of the single-stranded template nucleic acid to produce an anchor-template complex having a covalently attached 5' overhang;
- (b) sequencing the single-stranded template nucleic acid of the anchor-template complex having a covalently attached 5' overhang, thereby producing:
 - (i) a first sequence; and
 - (ii) an anchor-template-complement complex, comprising the anchor-template complex having a covalently attached 5' overhang of (a), and further comprising a synthetic nucleic acid strand complementary to the template nucleic acid, wherein the synthetic nucleic acid strand complementary to the template nucleic acid is hybridized to the template nucleic acid, and wherein the synthetic complementary nucleic acid strand complementary to the template nucleic acid is attached at its 5' end to the 3' end of the first end of the double-stranded nucleic acid anchor;
- (c) removing the synthetic complementary nucleic acid strand complementary to the template nucleic acid from the anchor-template-complement complex, thereby recovering the anchor-template complex having a covalently attached 5' overhang;
- (d) treating the anchor-template complex having a covalently attached 5'

overhang recovered in (c) with sodium bisulfite, thereby producing a sodium bisulfite-treated anchor-template complex;

- (e) sequencing the sodium bisulfite-treated anchor-template complex of (d), thereby producing a second sequence; and
 - (f) comparing the first sequence and the second sequence, where the presence of a cytosine in the second sequence indicates that the cytosine at that position in the template nucleic acid is methylated;
- thereby detecting a methylated cytosine in the template nucleic acid.

11. (currently amended) The method of claim 10, wherein the double-stranded nucleic acid anchor is attached ~~at its second end~~ to a solid support substrate.

12-18. (canceled).

19. (currently amended) A method for detecting a methylated cytosine in a template nucleic acid of known sequence, the method comprising:

- (a) providing a hairpin-template complex having a covalently attached 5' overhang, comprising:
 - (i) a hairpin nucleic acid, wherein the hairpin nucleic acid is self-complementary and has a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said recognition sequence is situated so that said cleavage site is before, at, or beyond the 3' end of the hairpin nucleic acid, and wherein said hairpin nucleic acid is a self-hybrid; and
 - (ii) a single-stranded template nucleic acid;wherein the 5' end of the hairpin nucleic acid is covalently attached to the 3' end of the single-stranded template nucleic acid to

produce a hairpin-template complex having a covalently attached 5' overhang;

- (b) treating the hairpin-template complex having a covalently attached 5' overhang with sodium bisulfite, thereby producing a sodium bisulfite-treated template nucleic acid;
 - (c) sequencing the sodium bisulfite-treated template nucleic acid of (b), thereby producing a sequence; and
 - (d) comparing the sequence of (c) and the known sequence, where the presence of a cytosine in the sequence of (c) indicates that the cytosine at that position is methylated;
- thereby detecting a methylated cytosine in the template nucleic acid of known sequence.

20. (currently amended) The method of claim 19, wherein the hairpin nucleic acid is attached to a solid support substrate.

21. (currently amended) A method for detecting a methylated cytosine in a template nucleic acid of known sequence, the method comprising:

- (a) providing an anchor-template complex having a covalently attached 5' overhang, comprising:
 - (i) a double-stranded nucleic acid anchor, wherein the double-stranded nucleic acid anchor comprises:
 - (A) a first end and a second end; and
 - (B) a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said cleavage site is situated so that said cleavage site is before, at, or beyond the 3' end of the first end of the double-stranded nucleic acid anchor, and

- (ii) a single-stranded template nucleic acid;
wherein the 5' end of the first end of the double-stranded nucleic acid anchor is covalently attached to the 3' end of the single-stranded template nucleic acid to produce an anchor-template complex having a covalently attached 5' overhang;
- (b) treating the anchor-template complex having a covalently attached 5' overhang with sodium bisulfite, thereby producing a sodium bisulfite-treated anchor-template complex;
- (c) sequencing the sodium bisulfite-treated anchor-template complex of (b), thereby producing a sequence; and
- (d) comparing the sequence of (c) and the known sequence, where the presence of a cytosine in the sequence of (c) indicates that the cytosine at that position in the template nucleic acid is methylated;
thereby detecting a methylated cytosine in the template nucleic acid.

22. (currently amended) The method of claim 21, wherein the double-stranded nucleic acid anchor is attached ~~at its second end~~ to a solid support ~~substrate~~.

23. (currently amended) A method for detecting a methylated cytosine in a template nucleic acid of known sequence, wherein non-methylated ~~one or more of the~~ cytosines in the template nucleic acid have been converted to uracil, the method comprising:

- (a) providing a hairpin-template complex having a covalently attached 5' overhang, comprising:
 - (i) a hairpin nucleic acid, wherein the hairpin nucleic acid is self-complementary and has a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said recognition sequence is situated so that said cleavage site is before, at, or beyond the 3' end

of the hairpin nucleic acid, and wherein said hairpin nucleic acid is a self-hybrid; and

(ii) a single-stranded template nucleic acid;

wherein 5' end of the hairpin nucleic acid is covalently attached to the 3' end of the single-stranded template nucleic acid to produce a hairpin-template complex having a covalently attached 5' overhang;

(b) sequencing the template nucleic acid, thereby producing a sequence; and

c) comparing the sequence of (b) and the known sequence, where the presence of a cytosine in the sequence of (b) indicates that the cytosine at that position is methylated;

thereby detecting a methylated cytosine in the template nucleic acid of known sequence.

24. (currently amended) The method of claim 23, wherein the hairpin nucleic acid is attached to a solid support ~~substrate~~.

25. (currently amended) A method for detecting a methylated cytosine in a template nucleic acid of known sequence, wherein non-methylated ~~one or more of the~~ cytosines in the template nucleic acid have been converted to uracil, the method comprising:

(a) providing an anchor-template complex having a covalently attached 5' overhang, comprising:

(i) a double-stranded nucleic acid anchor, wherein the double-stranded nucleic acid anchor comprises:

(A) a first end and a second end; and

(B) a first restriction site for a nicking endonuclease, said restriction site comprising a recognition sequence and a cleavage site, wherein said cleavage site is situated so that said cleavage site is before, at, or beyond

the 3' end of the first end of the double-stranded nucleic acid anchor; and

(ii) a single-stranded template nucleic acid;

wherein the 5' end of the first end of the double-stranded nucleic acid anchor is covalently attached to the 3' end of the single-stranded template nucleic acid to produce an anchor-template complex having a covalently attached 5' overhang;

(b) sequencing the anchor-template complex having a covalently attached 5' overhang, thereby producing a sequence; and

(c) comparing the sequence of (b) and the known sequence, where the presence of a cytosine in the sequence of (b) indicates that the cytosine at that position in the template nucleic acid is methylated;

thereby detecting a methylated cytosine in the template nucleic acid.

26. (currently amended) The method of claim 25, wherein the double-stranded nucleic acid anchor is attached at its second end to a solid support ~~substrate~~.

27. (new) The method of claim 11, wherein the double-stranded nucleic acid anchor forms part of an array comprising a plurality of double-stranded anchors distributed over the solid support.

28. (new) The method of claim 27, wherein the array comprises multiple copies of each individual double stranded nucleic acid anchor clustered at a single locus.

29. (new) A method for detecting a methylated cytosine in a pool of template nucleic acid which comprises:

(a) splitting the pool of template nucleic acid into two portions and treating one of said portions

of the template nucleic acid to produce a treated template nucleic acid portion wherein non-methylated cytosines are converted to uracil;

(b) separately sequencing each of the two template nucleic acid portions to generate sequence data for each template nucleic acid portion, wherein each of the portions is immobilized onto a separate microarray; and

(c) comparing sequence data generated for each microarray, wherein the presence of a cytosine at a position in the sequence data generated from the array comprising the treated template nucleic acid portion identifies a methylated cytosine at the position in the template nucleic acid.

30. (new) A method according to claim 29, wherein step (a) comprises splitting the pool of template nucleic acid into two portions and immobilising said portions of the template nucleic acid as single strands on two separate microarrays, and treating one microarray to convert non-methylated cytosines to uracil.

31. (new) A method according to claim 29, wherein each of said separate microarrays comprises immobilised anchor-template complexes, wherein each anchor-template complex has a covalently attached 5' overhang and comprises:

- (i) a double-stranded nucleic acid anchor comprising a first end and a second end; and
- (ii) a single-stranded template nucleic acid;

wherein the 5' end of the first end of the double-stranded nucleic acid anchor is covalently attached to the 3' end of the single-stranded template nucleic acid to form an anchor-template complex having a covalently attached 5' overhang and the anchor-template complex is attached to the solid substrate via the double-stranded nucleic acid anchor.

32. (new) The method of claim 29, wherein each microarray comprises multiple copies of

Serial No.: 10/537,186

Attorney Docket No.: 2713-1-016PCT/US

each individual double stranded nucleic acid anchor clustered at a single locus.